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Description

The invention relates generally to organophosphorous compounds, and more particularly, to organophosphorous compounds for synthesizing amino-derivatized polymers, especially oligonucleotides.

Genes and gene control regions can now be routinely characterized and studied at the molecular level. This is possible because of several recent advances in the technology associated with manipulating and modifying deoxyribonucleic acid (DNA). Of particular importance have been advances in DNA sequencing, Maxam and Gilbert, "Sequencing End-Labeled DNA with Base-Specific Chemical Cleavages," and Smith, "DNA Sequence Analysis by Primed Synthesis," pgs. 499-560 and 560-580, respectively, in Methods in Enzymology, Grossman and Moldave, eds., Vol. 65 (Academic Press, New York, 1980); the isolation of a large number of host restriction modification enzymes, Roberts, "Dictionary of Restriction Endonucleases," in Methods in Enzymology, Wu, ed., Vol. 68 (Academic Press, New York, 1979); and the construction of vectors for cloning and amplifying defined DNA sequences, e.g. Bolivar and Backman, "Plasmids of Escherichia coli as Cloning Vectors," in Methods in Enzymology, Wu, ed., Vol. 68 (Academic Press, New York, 1979).

Many of these new techniques require that DNA fragments or oligonucleotides be labeled or attached to polymer supports. DNA sequencing techniques and gene probes, which can be used to help locate natural genes of commercial or scientific importance, require the use of labeled oligonucleotides. Until recently, all DNA sequencing techniques relied on radioactive labels for distinguishing oligonucleotide fragments separated by electrophoresis. Radioactive labels are highly sensitive, and can be incorporated without steric hinderance, or other chemical side effects. However, their use poses a laboratory health hazard, which requires that they receive special handling and disposal. Moreover, their use is not amenable for rapid automatic sequencing of oligonucleotides, as nucleoside-specific radioactive labels are not available for practical identification of different nucleotide bases, and radiation detection techniques such autoradiography and scintillation counting are too time consuming. As a consequence, other non-radioactive labeling-techniques have been sought, such as fluorescent and colorimetric labeling, which depend on the ability to covalently link a fluorescent or chromogenic molecule to an oligonucleotide.

Chu et al, in "Derivatization of Unprotected Poly nucleotides," Nucleic Acids Research, Vol.11, pgs. 6513-6529(1983), disclose a method for attaching amines to the terminal 5'-phosphates of oligonucleotides. One object of the method is to provide a means for attaching organic labeling molecules to oligonucleotides by way of an amine linkage. The method involves treating the oligonucleotides with a carbodiimide.

Chollet and Kawashima, in "Biotin-Labeled Synthetic Oligodeoxyribonucleotides: Chemical Synthesis and Uses as Hybridization Probes," Nucleic Acids Research, Vol.13, pgs. 1529-1541 (1985), disclose the use of the method of Chu et al to attach biotin to the 5'-phosphate of an oligonucleotide. The reported yields of 50-70% are below that needed for use in automatic synthesizers, and the carbodiimide can cause unwanted modifications to oligonucleotide bases in the course of the reaction.

Smith et al, in "Synthesis of Oligonucleotides Containing an Aliphatic Amino Group at the 5' Terminus: Synthesis of Fluorescent DNA Primers for Use in DNA Sequence Analysis," Nucleic Acids Research, Vol.13, pgs. 2399-2412 (1985), disclose a protected amino-derivatized nucleoside phosphoramidite for linking fluorescent or colorimetric tags to oligonucleotide fragments. While the linker is highly useful for attaching base-specific labels to the 5' terminus of oligonucleotides, the protected-amine phosphoramidite is not readily purified.

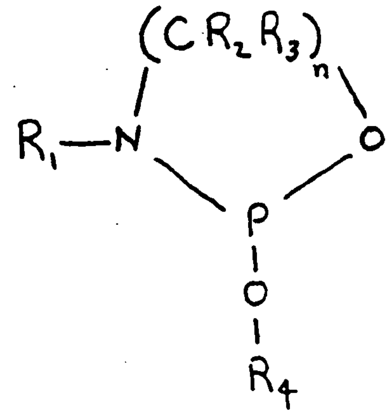
Connolly and Rider, in "Chemical Synthesis of Oligonucleotides Containing a Free Sulphydryl Group and Subsequent Attachment of Thiol Specific Probes," Nucleic Acids Research, Vol. 13, pgs. 4485-4502 (1985), disclose the synthesis of oligonucleotides having a trityl-protected sulphur attached via a two, three, or six carbon chain to the 5' phosphate of the oligonucleotide.

Apart from linking labeling agents to oligonucleotides, there is great interest in immobilizing various molecules on polymer supports, such as catalysts, enzymes, microorganisms, affinity reagents, immunoadsorbents, and the like, for both preparative and analytical uses, e.g. Schott, Affinity Chromatography (Marcel Dekker, Inc., New York,1984), and Mosbach, ed., Methods in Enzymology, Vol.44, "Immobilized Enzymes," (Academic Press, New York, 1976). Of particular interest in this field are means for immobilizing molecules and cells by covalent bonds.

The compounds of the invention include novel linking agents comprising 2-substituted-3-protected-1,3,2-oxazaphosphacycloalkanes and their phosphoramidite precursors. The compounds of the invention further include conjugates of the above mentioned linking agents with oligonucleotides, conjugates of the above mentioned linking agents with polymer supports, and conjugates comprising dyes linked to oligonucleotides by the above mentioned linking agents. The present invention relates to compounds that are useful for linking organic moieties, such as fluorescent and chromogenic dyes, to DNA fragments and

oligonucleotides, particularly single-stranded DNA and RNA, and for linking DNA fragments, oligonucleotides, proteins, and the like to polymer supports. The compounds and their conjugates are useful in automated and manual DNA and RNA synthesis and sequence analysis, construction of gene probes, affinity techniques, and the like. In particular, the cyclic embodiments of the linking agents of the invention advantageously overcome deficiencies associated with currently available linking methods by providing more readily purified linking agents.

The linking compounds of the present invention include 2-substituted-3-protected-1,3,2-oxazaphosphacycloalkanes defined by the formula:



Formula I

wherein:

n is 2,3 or 4, preferably 2 or 3, and most preferably is 2.

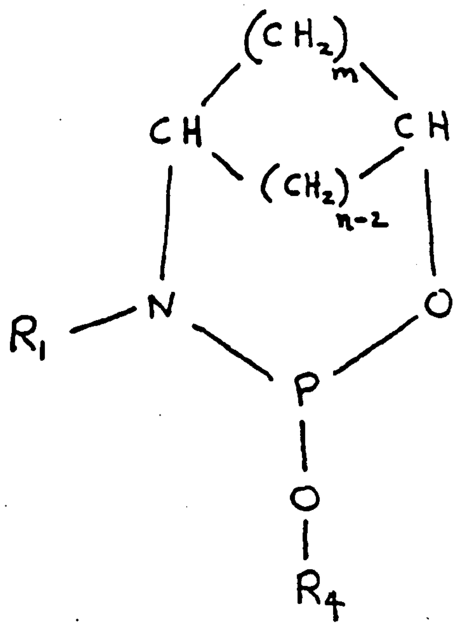
R_1 is either acid-labile or base-labile amino protection groups, e.g. as described by Greene, in Protective Groups in Organic Synthesis (John Wiley & Sons, New York, 1981), chapter 7,. The base-labile protection groups, are, trihaloacetyl, acetoacetyl, and fluorenylmethyl carbamate, particularly 9-fluorenylmethyl carbamate and 9-(2-sulfo)-fluorenylmethyl carbamate, and most preferably trifluoroacetyl. The acid-labile protection groups are trityl, and C_1 to C_3 alkoxy substituted trityl, particularly 4-monomethoxytrityl and 4,4'-dimethoxytrityl.

R_2 and R_3 are chosen so that (1) the likelihood that they sterically hinder the cyclization of the compound of Formula I is minimized, (2) the ring electron density of the heterocycle of Formula I is reduced, because it is thought that this will enhance the reactivity or the N-P bond in the compound of Formula I to hydroxyl groups, and (3) the molecular weight of the compound of Formula I is minimized to increase the likelihood that it can be purified by distillation. Each of R_2 and R_3 is hydrogen; C_1 to C_6 alkyl; mono-, di- or trihalomethyl, halo-, cyano-, sulfo- or nitro- substituted C_1 to C_6 alkyl; cyano, halo or nitro and more preferably R_2 and R_3 are hydrogens.

R_4 is alkyl, alkenyl, aryl, aralkyl, or cycloalkyl containing up to 10 carbon atoms. More preferably, R_4 is C_1 to C_6 alkyl; electron-withdrawing beta-substituted ethyl, particularly beta-trihalomethyl-, beta-cyano-, beta-sulfo-, beta-nitro-substituted ethyl,; electron-withdrawing substituted phenyl, particularly halo-, sulfo-, cyano-, or nitro-, substituted phenyl; or electron-withdrawing substituted phenylethyl, particularly halo-, nitro-, sulfo-; or cyano-substituted phenylethyl Most preferably, R_4 is methyl, beta-cyanoethyl, or 4-nitrophenylethyl.

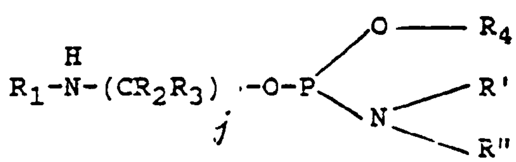
C_1 to C_6 alkyl denotes straight-chain and branched-chain alkyl groups e.g. methyl, ethyl, propyl, isopropyl, tert-butyl, isobutyl, sec-butyl, neopentyl, tert-pentyl, and the like. "Electron-withdrawing" denotes the tendancy of a substituent to attract valence electrons of the molecule of which it is apart, i.e. it is electronegative.

A special case of The above-described linking agent includes bicyclic compounds defined by the formula:



Formula II

wherein m is 1, 2 or 3; more preferably m is 1 or 2; and most preferably m is 1; n is 2 or 3; and R_1, and R_4 are as described for formula I. The linking compounds of the invention also include the phosphoramidite precursors to the above 2-substituted-3-protected-1,3,2-oxazaphosphacyloalkanes, the precursors being defined by the formula:



Formula III

wherein

j is an integer from 2 to 10, preferably 2, 3 or 4 and more preferably j is 2 or 3 (it is believed that this latter range results in a precursor possessing the most favourable steric configuration and cyclization);

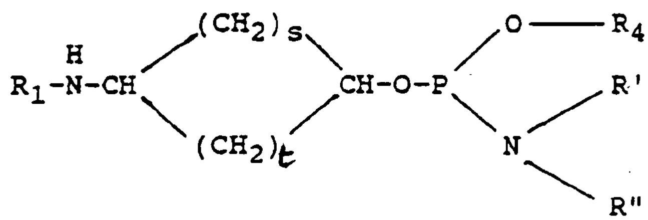
R_1 is an amino protection group, preferably R_1 is trihaloacetyl, acetoacetyl, fluorenylmethyl carbamate, trityl or C_1 to C_3 alkoxy substituted trityl and more preferably R_1 is trifluoracetyl, 9-(2-sulpho)-fluorenylmethyl carbamate, 9-fluorenylmethyl carbamate, 4-monomethoxytrityl or 4,4'-dimethoxytrityl;

each of R' and R" is alkyl, aryl, aralkyl, cycloalkyl, and cycloalkylalkyl containing up to 10 carbon atoms. Preferably each of R' and R", when the above phosphoramidites are used directly as linking agents, are sterically hindering lower alkyls which enhance the chemical stability of the phosphoramidites, and hence their shelf lives. Such sterically hindering lower alkyls include propyl, isopropyl, isobutyl, sec.butyl or t.butyl, neopentyl, tert-pentyl, isopentyl, sec-pentyl, and the like. Most preferably, when the above phosphoramidites are used as precursors to the above-described oxazaphospha-heterocycle, each of R' and R" is isopropyl;

or R' and R" together form an alkylene chain containing up to 5 carbon atoms in the principal chain and a total of up to 10 carbon atoms with both terminal valence bonds of the chain being attached to the valence bonds of the chain being attached to the nitrogen atom to which R' and R" are attached ; or R' and

R" together with the nitrogen atom to which they are attached form a saturated nitrogen heterocycle which may contain one or more additional heteroatoms from the group consisting of nitrogen, oxygen, and sulfur. Preferably, R' and R" together with the nitrogen atom to which they are attached is pyrrolidino, morpholino, or piperidino, and more preferably is morpholino.

The phosphoramidite precursors to the above-described 2-substituted-3-protected-1,3,2-oxazaphosphacycloalkanes include substituted lower cycloalkanes defined by the formula:



Formula IV

wherein

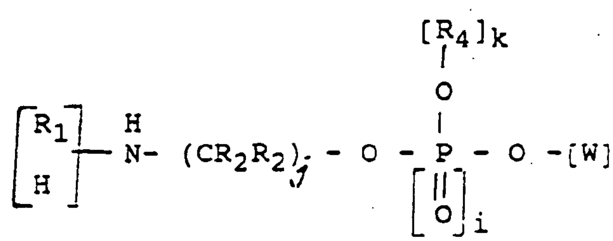
s is in the range of 0 to 8, t is in the range of 0 to 8, and s+t is in the range of 1 to 8, preferably t is 0, 1 or 2 and s is 1, 2 or 3, more preferably t is 0 and s is 1 or 2, and most preferably t is 0 and s is 1;

R_1 is as described for the cycloalkane of formula III;

R_4 is as described for formula I; and

each of R' and R" is alkyl, aryl, aralkyl, cycloalkyl or cycloalxylalkyl containing up to 10 carbon atoms or R' and R" together with the nitrogen atom to which they are attached is morpholino, pyrrolidino or piperidino.

Conjugates of the present invention include triester phosphite and triester and diester phosphate compounds of the formula



Formula V

wherein:

i is 0 or 1 (where 1 = 0 indicates phosphite, and i = 1 indicates phosphate);

j is an integer from 2 to 10;,

k is (-where k = 0 indicates diester, and k = 1 indicates triester) whenever i equals 0, or k equals 0 or l whenever i equals 1;

R_1 is as described for formula III;

R_2, R_3, and R_4 are as described for formula I; and W represents an oligonucleotide, a polymer support, or an oligonucleotide linked to a polymer support. Oligonucleotides include fragments of single-stranded and double-stranded RNA, and fragments of single- and double-stranded DNA. Preferably the linking agent is conjugated to the terminal 5' carbon of an oligonucleotide, the terminal 3' carbon of an oligonucleotide, or the terminal 2' carbon of RNA. More preferably, the linking agent is conjugated to the terminal 5' carbon of an oligonucleotide, and most preferably the linking agent is conjugated to the terminal 5' carbon of a fragment of single-stranded DNA.

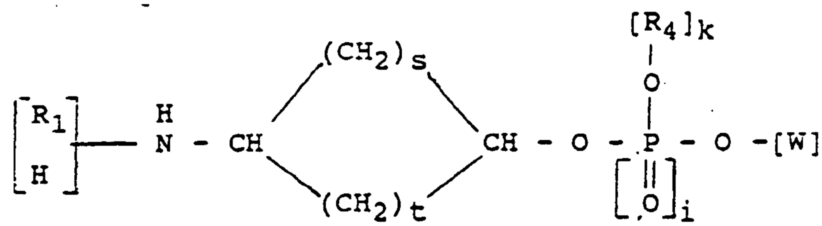
Polymer supports may have a variety of forms and compositions. The polymer support can be derived from naturally occurring materials, naturally occurring materials which are synthetically modified, and synthetic materials. Of particular interest are polysaccharides, particularly crosslinked polysaccharides, such as agarose, which is available as Sepharose, dextran, available as Sephadex and Sephacyl, cellulose, starch and the like (Sepharose, Sephadex, and Sephacyl being trademarked products of Pharmacia Fine Chemicals). Other materials include polyacrylamides, polystyrenes, polyvinyl alcohols, copolymers of hydroxyethyl methacrylate and methyl methacrylate, silicones, teflons glasses, cells, or the like. In addition to solid

supports in the form of particles and the like, the polymer support may also be in the form of liquid particles comprising a lipophilic or amphiphilic membrane, which serves to contain an internal fluid and define a space. Such particles include vesicles, cells, and liposomes. Preferably A′ represents an insoluble polymer support having hydroxyl functionalities. The linking agents of the invention are attached to polymer supports having hydroxyl functionalities by following the procedures generally described below for attaching the linking agents to oligonucleotides.

The bracket on the lefthand side of Formula V (enclosing H and R_1) indicates that this embodiment includes both the protected and deprotected forms of the compound.

Oligonucleotides are linked to polymer supports by standard techniques of affinity chromatography or, for example, by linking means disclosed by Caruthers et al. in U.S. Patents 4,458,066 and 4,415,732 or the like.

The triester phosphite and triester and diester phosphate conjugates of the present invention further include compounds of the formula:



Formula VI

wherein

R_1 is as described for formula III;

s is in the range of 0 to 8, t is in the range of 0 to 8, and s+t is in the range of 1 to 8;

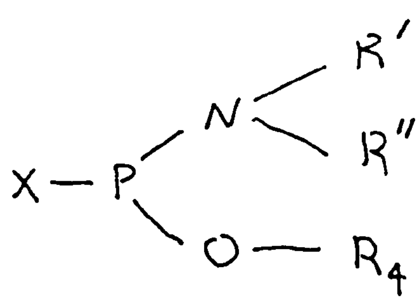
R_4 is C_1 to C_6 alkyl; beta-trihalomethyl-, beta-nitro-, beta-sulfo-, or beta-cyano-substituted ethyl; halo-, nitro-, sulfo- or cyano-substituted phenyl; or halo-, nitro-, sulfo- or cyano- substituted phenylethyl;

k is 1 whenever i is 0, or k is 0 or 1 whenever i is 1; and

W is an oligonucleotide, a polymer support, or an oligonucleotide linked to a polymer support.

Generally the triester phosphate compounds of the invention are readily obtained from the above-defined phosphite conjugates by oxidation, e.g. with the use of I_2 in water, 2,6-lutidine and tetrahydrofuran. Oxidation is extremely rapid (1-2 minutes).

The diester phosphate conjugates of the invention are readily obtained from the above-defined triester phosphates by standard techniques, for example when R_4 is methyl, the diester phosphates are obtained from the triester phosphates by treatment with thiophenol/triethylamine for about 30 minutes. The general procedure for synthesizing the phosphoramidite precursors of Formulas III and IV comprises the following steps. Halo-substituted-N,N-di-substituted-O-substituted phosphine, defined by the formula:



wherein X is a halogen, usually chloro, and R′, R″, and R_4 are as indicated above, is reacted with an amino-protected alcohol amine defined by the formula:

$$R_1-\overset{H}{N}-(CR_2R_3)_n-OH$$

wherein R_1, R_2, and R_3 are as indicated above, in an aprotic solvent, such as dichloromethane, or the like, containing a non-nucleophilic base, for example a trialkylamine, such as N,N-diisopropylethyl amine, or the like, which absorbs the halogen acid released during the reaction. Preferably the reaction takes place under an inert atmosphere, such as argon. Acid conditions in the reaction mixture should be avoided as acid causes the amine of the phosphoramidite product to protonate, and thereby become reactive. The non-nucleophilic base reduces the likelihood of side reactions between the base and activated phosphoramidites.

Whenever the alkyl moiety, i.e. $-(CR_2R_3)_n-$, of the amino-protected alcohol amine is cycloalkyl, e.g. as in Formula IV, the amide or carbamate moiety of the alcohol amine is preferably in the cis configuration with the hydroxy; otherwise, formation of the oxazaphosphaheterocycle becomes unlikely, or even impossible, because of the spacial separation of the two groups.

After reacting the above materials, the reaction mixture, hereinafter referred to as the first reaction mixture, is washed with a mildly basic solution to remove salts of the non-nucleophilic base. Finally, the first reaction mixture is dried, e.g. with $MgSO_4$, Na_2SO_4, or the like, to give the phosphoramidite precursor.

The heterocycles of Formulas I and II are then obtained by heating the appropriate precursor represented by Formulas III or IV, respectively, to form a second reaction mixture, and then separating the heterocycle from the mixture. The necessary amount of heating, i.e. temperature and duration, varies with different embodiments of the invention, preferably heating includes raising the precursor to a temperature within the range of about 25 to 250 °C, more preferably from about 25 to 150 °C, and most preferably from about 25 to 100 °C. The choice of method of separation depends on the nature of the substituent groups, R_1, R_2, R_3, and R_4. For example, as a rough approximation when the aggregate molecular weight of the substituents is sufficiently low, the steps of heating and separating can be accomplished by distilling. Other methods of separation include crystallization and chromatography. Preferably conjugates of oligonucleotides and linking agents of the invention are formed by attaching the linking agent to oligonucleotides synthesized by the solid phase synthetic methods developed by Caruthers and his coworkers, e.g. Caruthers et al., pgs. 1-17, in Genetic Engineering, Vol. 4, Setlow and Hollaender, Eds. (Plenum Press, New York, 1982), and Caruthers et al., U.S. Patent 4,458,066. Attachment of the linking agent occurs as the final step in the synthetic process; that is, the linking agent is attached to the oligonucleotide as if it were a nucleotide subunit in the Caruthers et al. method.

The following examples serve to illustrate the present invention. The concentrations of reagents, temperatures, and values of other variable parameters are only to exemplify the application of the present invention and are not to be considered as limitations thereof.

EXAMPLE I. Synthesis of the phosphoramidite precursor of 2-methoxy-3-trifluoroacetyl-1,3,2-oxazaphosphacyclopentane

Chloro-N,N-diisopropylaminomethoxy phosphine (5.0 ml, available form Aldrich Chemical Co., Milwaukee, WI) was added dropwise at 0 °C to a stirred solution of N-(2-hydroxyethyl)-2,2,2-trifluoroacetamide (3.9 g) and diisopropylethylamine (5.0 ml) in dichloromethane (about 40 ml) under argon. (N-(2-hydroxyethyl)-2,2,2-trifluoroacetamide is synthesized following the procedures disclosed by Lazarus and Benkovic in J. Am. Chem. Soc., Vol. 101, pgs. 4300-4312 (1979): Ethyl trifluoroacetate (56.8g, 0.4 mol) in 50 mL of chloroform is added dropwise to a stirred solution of 24.4 g (0.4 mol) of ethanolamine in 50 mL of chloroform. The solution is stirred at room temperature for 5 h, rotary evaporated to remove the solvent, and distilled at 115 °C (4.3 Torr) to give 58.5 g of oil that crystallized upon scratching.) After stirring at room temperature for 0.5 hours the reaction mixture was washed twice with 0.2 M potassium carbonate solution and once with brine, dried ($MgSO_4$), and concentrated under reduced pressure to give N-(2-(N',N'-diisopropylaminomethoxyphosphinyloxy)ethyl)-2,2,2-trifluoroacetamide as a colorless liquid (7.77 g).

^{1}H-NMR (CD_2Cl_2): δ 3.6 (6H, m), 3.4 (3H, d, J = 12), 1.2 (12H, d, J = 6.5)

^{31}P-NMR (CD_2Cl_2, ^{1}H-decoupled): δ149(s)

EXAMPLE II. Synthesis of the phosphoramidite precursor of 2-methoxy-3-trifluoroacetyl-1,3,2-oxazaphosphacyclohexane

Chloro-N,N-diisopropylaminomethoxy phosphine (3.7 ml) was added dropwise at 0 °C to a stirred solution of N-(3-hydroxypropyl)-2,2,2-trifluoroacetamide (2.9 g, synthesized from 3-amino-1-propanol and ethyltrifluoroacetate in a manner similar to that disclosed by Lazarus and Benkovic, J. Amer. Chem. Soc., Vol.101, pgs. 4300-4312 (1979)) and diisopropylethylamine (3.7 ml) in dichloromethane (about 20 ml) under argon. After stirring at room temperature for 3 hours, the reaction mixture was poured into hexane (100 ml) and stirred. The mixture was allowed to settle and the supernatant was separated and concentrated under reduced pressure to give N-(3-(N',N'-diisopropylaminomethoxyphosphinyloxy)propyl)-2,2,2-trifluoroacetamide as a colorless liquid (5.2 g).

^{31}P-NMR (CH_2Cl_2, ^{1}H decoupled): δ149 (s)

EXAMPLE III. Synthesis of 2-methoxy-3-trifluoroacetyl-1,3,2-oxazaphosphacyclopentane

N-(2-(N',N'-diisopropylaminomethoxyphosphinyloxy)-ethyl)-2 2,2-trifluoroacetamide (7.7 g) was distilled (58-59 °C at 0.8 Torr) to quantitatively yield 2-methoxy-3-trifluoroacetyl-1,3,2-oxazaphosphacyclopentane as a colorless liquid.

IR (film):	1705, 1420, 1230, 1200, 1160, 1020, 965 cm^{-1}
^{1}H-NMR (CD_2Cl_2):	δ 4.45 (2H, m), 3.65 (2H, m), 3.60 (3H, d, J=12)
^{31}P-NMR (CD_2Cl_2, ^{1}H-decoupled):	δ132(s), 125 (q, J=61)
MS:	m/e 217 (M^+), 197, 148, 136, 123, 120, 109, 92, 79, 70(100), 69, 62

EXAMPLE IV. Attaching 2-methoxy-3-trifluoroacetyl-1,3,2-oxazaphosphacyclopentane to the 5' terminus of an oligonucleotide

Attachment of 2-methoxy-3-trifluoroacetyl-1,3,2-oxazaphosphacyclopentane to a 5' hydroxyl of an oligonucleotide was performed on an Applied Biosystems 380A DNA synthesizer (Applied Biosystems, Foster City, CA), or comparable instrument. Caruthers et al, U.S. Patent 4,458,066; Caruthers et al, U.S. Patent 4,415,732; and Caruthers et al, "New Methods for Synthesizing Deoxyoligonucleotides," in Genetic Engineering, Vol. 4, pgs. 1-17 (Plenum Press, New York, 1982) provide detailed descriptions of the chemistry used by the Applied Biosystems 380A DNA synthesizer. Accordingly, these references are incorporated by reference for those descriptions. 2-Methoxy-3-trifluoroacetyl-1,3,2-oxazaphosphacyclopentane was used as a 0.2 M acetonitrile solution in combination with 0.5 M tetrazole/acetonitrile solution to form an activated reagent in the synthesis cycle. The normal synthesizer cycle was modified only during the addition of the activated reagent in the following manner. The activated reagent was added twice with 1 hour wait times after each addition. The coupling yields were about 95%. Normal deprotection with thiophenol/triethylamine and then ammonium hydroxide gave a 5'-aminoethylphosphate oligonucleotide. Similar yields were obtained when the activated reagent comprised an acetonitrile solution containing 0.2 M 2-methoxy-3-trifluoroacetyl-1,3,2-oxazaphosphacyclopentane and 0.1 M 4-dimethylaminopyridine. In this case the modified activator reagent was added once, and allowed to react for about 15 minutes.

EXAMPLE V. Attaching 2-methoxy-3-trifluoroacetyl-1,3,2-oxazaphosphacyclopentane to the 3' terminus of an oligonucleotide

Attachment is accomplished in substantially the same manner as described in Example IV, except the oligonucleotide is synthesized in the 3' direction in accordance with the procedure generally described in Caruthers et al, U.S. Patent 4,458,066 (Roughly the difference is that the oligonucleotide is synthesized from 5' N,N-diisopropylaminophosphoramidites of 3'-protected nucleosides instead of 3' N,N-diisopropylaminophosphoramidites of 5'-protected nucleosides. Alternatively, the oligonucleotide is synthesized in the 3' direction using the phosphotriester method of Khorana and Itakura (i.e., Korana, Science, Vol. 203, pgs. 614-625 (1979); Itakura et al. J. Biol. Chem., Vol. 250, pgs. 4592-4600, both of these references being incorporated by reference), or its modification by others, for example Letsinger and Mahaderan, J. Am. Chem. Soc., Vol. 187, pgs. 3526- (1965). In any case the linking agent is attached as a final addition in place of a nucleotide.

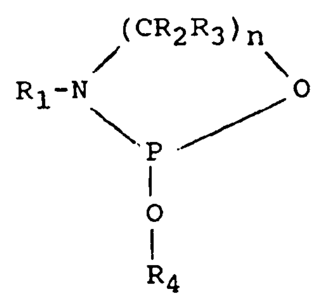
EXAMPLE VI. Attaching Fluorescein isothiocyanate (FITC) to a 5' aminoethylphosphate oligonucleotide

A dimethylformamide solution of fluorescein-6-isothiocyanate (25 microliters at a concentration of 10 mg/ml, e.g. available form Molecular Probes, Inc., Junction City, OR) was added to a solution of 5'-aminoethylphosphate TCCCAGTCACGACGTT (0.020 micromole, unpurified material being made on an

Applied Biosystems 380A DNA synthesizer; here T = thymidine, C = cytidine, G = guanosine, and A = adenosine) in water (200 microliters) and 1 M $NaHCO_3/Na_2CO_3$ buffer, pH 9.0 (25 microliters). The resulting solution was stored in the dark at room temperature for at least 6 hours. To remove the unconjugated dye, the reaction mixture was passed through an equilibrated 10 ml Sephadex (trademark of Pharmacia Fine Chemicals) G-25 (medium) column with water. The band of colored material in the excluded volume was collected. The crude 5'-fluorescein aminoethylphosphate oligonucleotide was purified by HPLC (e.g. Perkin-Elmer Series 4, or comparable instrument) on a Vydac C18 column (No. 218TP54), or the like, in a linear gradient of 10-20% acetonitrile/0.1 M triethylammonium acetate, pH 7.0.

Claims

1. A compound of the formula:



wherein:-

n is 2, 3 or 4;

R_1 is trihaloacetyl, acetoacetyl, fluorenylmethyl carbamate, trityl or C_1 to C_3 alkoxy-substituted trityl;

each of R_2 and R_3 is hydrogen; C_1 to C_6 alkyl; mono-, di- or trihalomethyl-, halo-, cyano-, sulfo- or nitro-substituted C_1 to C_6 alkyl; cyano, halo or nitro; and

R_4 is alkyl, alkenyl, aryl, aralkyl or cycloalkyl containing up to 10 carbon atoms.

2. A compound according to claim 1, wherein n is 2 or 3.

3. A compound according to claim 1 or claim 2, wherein R_4 is C_1 to C_6 alkyl; beta-trihalomethyl-, beta-nitro-, beta-sulfo- or beta-cyano- substituted ethyl; halo-, nitro-, sulfo- or cyano-substituted phenyl; or halo-, nitro-, sulfo- or cyano-substituted phenylethyl.

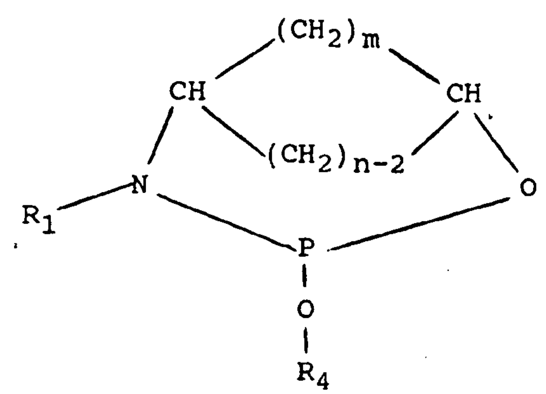
4. A compound according to claim 3, wherein R_4 is methyl.

5. A compound according to any one of claims 1 to 4, wherein R_1 is trifluoroacetyl, 9-(2-sulfo)-fluorenylmethyl carbamate or 9-fluorenylmethyl carbamate.

6. 2-Methoxy-3-trifluoroacetyl-1,3,2-oxazaphosphacyclopentane.

7. 2-Methoxy-3-trifluoroacetyl-1,3,2-oxazaphosphacyclohexane.

8. A bicyclic compound of the formula:



wherein:

m is 1, 2 or 3;

n is 2 or 3;

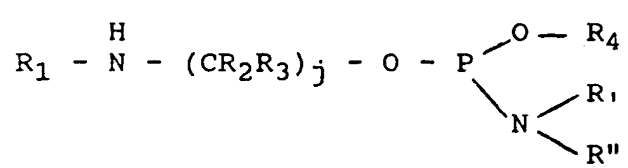
R_1 is trihaloacetyl, acetoacetyl, fluorenylmethyl carbamate or trityl or C_1 to C_3 alkoxy-substituted trityl; and

R_4 is alkyl, alkenyl, aryl, aralkyl or cycloalkyl containing up to 10 carbon atoms.

9. A compound according to claim 8, wherein R_4 has the meaning specified in claim 3.

10. A compound according to claim 8 or claim 9, wherein R_1 has the meaning specified in claim 5.

11. A compound of the formula:



wherein:

j is an integer from 2 to 10;

R_1 is an amino protection group;

each of R_2 and R_3 is hydrogen; C_1 to C_6 alkyl; mono-, di- or trihalomethyl-, halo-, cyano-, sulfo- or nitro-substituted C_1 to C_6 alkyl; cyano, halo or nitro; and

R_4 is alkyl, alkenyl, aryl, aralkyl or cycloalkyl containing up to 10 carbon atoms;

each of R' and R" is alkyl, aryl, aralkyl, cycloalkyl, or cycloalkylalkyl containing up to 10 carbon atoms; or

R' and R" together form an alkylene chain containing up to 5 carbon atoms in the principal chain and a total of up to 10 carbon atoms with both terminal valence bonds of the chain being attached to the nitrogen atom to which R' and R" are attached; or R' and R" together with the nitrogen atom to which they are attached form a saturated nitrogen heterocycle.

12. A compound according to claim 11, wherein R_1 is trihaloacetyl, acetoacetyl or fluorenylmethyl carbamate and R_4 is C_1 to C_6 alkyl; beta-trihalomethyl-, beta-nitro-, beta-sulfo- or beta-cyano-substituted ethyl; halo-, nitro-, sulfo- or cyano-substituted phenyl; or halo-, nitro-, sulfo- or cyano-substituted phenylethyl.

13. A compound according to claim 12, wherein R_1 is trifluoroacetyl, 9-(2-sulfo)-fluorenylmethyl carbamate or 9-fluorenylmethyl carbamate.

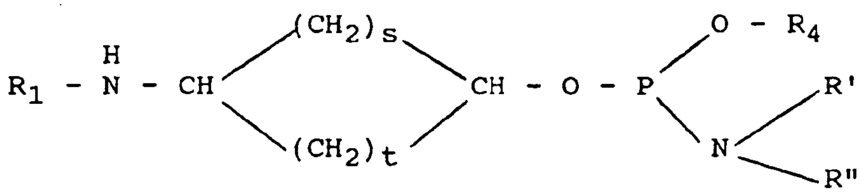
14. A compound according to any one of claims 11 to 13, wherein each of R' and R" is propyl, isopropyl, isobutyl, sec.butyl or t.butyl or R' and R" together with the nitrogen atom to which they are attached is morpholino, pyrrolidino, or piperidino.

15. A compound according to claim 14, wherein each of R' and R" is isopropyl or R' and R" together with the nitrogen atom to which they are attached is morpholino.

16. A compound according to claims 14 or claim 15, wherein R_1 is trityl or C_1 to C_3 alkoxy-substituted trityl.

17. A compound according to claim 16, wherein R_1 is 4-monomethoxytrityl or 4,4'-dimethoxytrityl.

18. A cycloalkane of the formula:



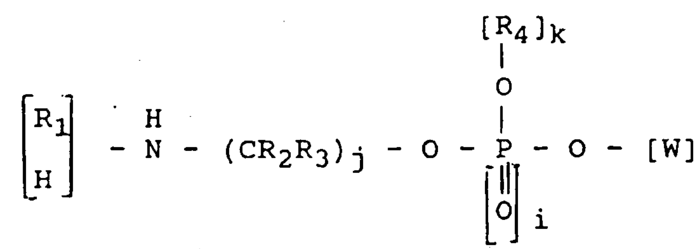
wherein:
s is in the range of 0 to 8, t is in the range of 0 to 8, and s+t is in the range of 1 to 8;
R_1 is an amino protection group;
R_4 is alkyl, alkenyl, aralkyl, or cycloalkyl containing up to 10 carbon atoms;
each of R' and R" is alkyl, aryl, aralkyl, cycloalkyl or cycloalkylalkyl containing up to 10 carbon atoms or R' and R" together with the nitrogen atom to which they are attached is morpholino, pyrrolidino or piperidino.

19. A compound according to claim 18, wherein R_1 is trihaloacetyl, acetoacetyl or fluorenylmethyl carbamate and R_4 is C_1 to C_6 alkyl, beta-trihalomethyl-, beta-nitro-, beta-sulfo- or beta-cyano-substituted ethyl; halo-, nitro-, sulfo- or cyano-substituted phenylethyl.

20. A compound according to claim 18, wherein R_1 is trityl or C_1 to C_3 alkoxy-substituted trityl.

21. A compound according to claim 20, wherein R_1 is 4-monomethoxytrityl or 4,4'-dimethoxytrityl.

22. A compound of the formula:

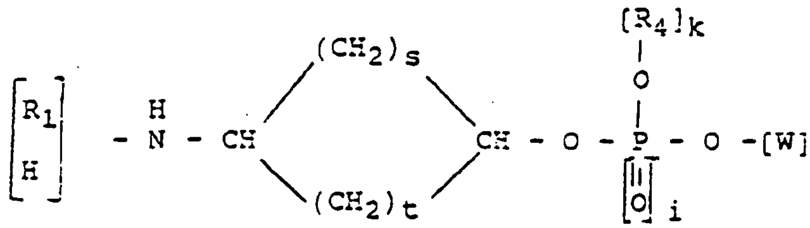


wherein:
i is 0 or 1;
j is an integer from 2 to 10;
k is 1 whenever i is 0, or k is 0 or 1 whenever i is l;
R_1 is an amino protection group;
each of R_2 and R_3 is hydrogen; C_1 to C_6 alkyl; mono, di- or trihalomethyl-, halo-, cyano-, sulfo-or nitro-substituted C_1 to C_6 alkyl; cyano, halo or nitro;
R_4 is alkyl, alkenyl, aryl, aralkyl, or cycloalkyl containing up to 10 carbon atoms; and
W represents an oligonucleotide, a polymer support, or an oligonucleotide linked to a polymer support.

23. A compound according to claim 22, wherein each of R_2 and R_3 is hydrogen and R_4 is C_1 to C_6 alkyl;

beta-trihalomethyl-, beta-nitro-, beta-sulfo- or beta-cyano- substituted ethyl; halo-, nitro-sulfo- or cyano-substituted phenyl; or halo-, nitro-, sulfo- or cyano-substituted phenylethyl.

24. A cyclic compound of the formula:



wherein:

R_1 is an amino protection group;

s is in the range of 0 to 8, t is in the range of 0 to 8, and s+t is in the range of 1 to 8;

R_4 is C_1 to C_6 alkyl; beta-trihalomethyl-, beta-nitro-, beta-sulfo-, or beta-cyano-substituted ethyl; halo-, nitro-, sulfo- or cyano-substituted phenyl; or halo-, nitro-, sulfo- or cyano- substituted phenylethyl;

k is 1 whenever i is 0, or k is 0 or 1 whenever i is 1; and

W is an oligonucleotide, a polymer support, or an oligonucleotide linked to a polymer support.

25. A method of synthesizing 2-lower alkoxy-3-protected-1,3,2-oxazaphosphacycloalkanes, the method comprising the steps of:

providing a phosphoramadite precursor of claim 11 or 18;

heating the phosphoramidite precursor to form a reaction mixture containing a 2-lower alkoxy-3-protected-1,3,2-oxazaphosphacycloalkane; and

separating the 2-lower alkoxy-3-protected-1,3,2-oxazaphosphacycloalkane from the reaction mixture.

26. A method according to claim 25, wherein the phosphoramadite precursor is provided by:

reacting a halo-substituted-N,N-di-substituted-lower alkoxy phosphine with an amino-protected alcohol amine in an aprotic solvent to form a first reaction mixture containing the phosphoramidite precursor; and

separating the phosphoramidite precursor from the first reaction mixture.

27. A method according to claim 25 or claim 26, wherein the step of heating includes heating to a temperature in the range of 25-250° C.

28. A method according to any one of claims 25 to 27, wherein the step of separating the 2-lower alkoxy-3-protected-1,3,2-oxazaphosphacycloalkane includes distilling.

29. A method according to any one of claims 25 to 28, wherein the 2-lower alkoxy-3-protected-1,3,2-oxazaphosphacycloalkaneis 2-methoxy-3-trifluoroacetyl-1,3,2-oxazaphosphacyclopentane or 2-methoxy-3-trifluoroacetyl-1,3,2-oxazaphospha cyclohexane.

30. A method of labelling an oligonucleotide, comprising the steps of:

reacting a compound of claim 1, 8, 11 or 18 with an unprotected hydroxyl of the oligonucleotide to form a linker oligonucleotide conjugate, the linker oligonucleotide conjugate having a protected amine;

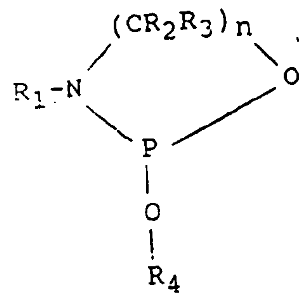
deprotecting the protected amine; and

reacting a label with the deprotected amine.

31. A method according to claim 30, further including the step of synthesising the oligonucleotide by a solid phase synthesis, and wherein the step of reacting the compound of claim 1, 8, 11 or 18 is accomplished as a final addition step in the solid phase synthesis.

Revendications

1. Composé représenté par la formule :



dans laquelle :
- n vaut 2, 3 ou 4 ;
- R_1 représente trihaloacétyle, acétoacétyle, fluorénylméthyl carbamate, trityle ou trityle substitue par alcoxy en C_1 à C_3 ;
- R_2 et R_3 représentent chacun hydrogène ; alkyle en C_1 à C_6 ; alkyle en C_1 à C_6 substitué par mono-, di- ou trihalométhyle, halo, cyano, sulfo ou nitro ; cyano, halo ou nitro ; et
- R_4 représente alkyle, alcényle, aryle, aralkyle ou cycloalkyle contenant jusqu'à 10 atomes de carbone.

2. Composé selon la revendication 1, dans lequel n vaut 2 ou 3.

3. Composé selon la revendication 1 ou la revendication 2, dans lequel R_4 représente alkyle en C_1 à C_6 ; éthyle substitué par bêta-trihalométhyle, bêta-nitro, bêta-sulfo ou bêta-cyano ; phényle substitué par halo, nitro, sulfo ou cyano ; ou phényléthyle substitué par halo, nitro, sulfo ou cyano.

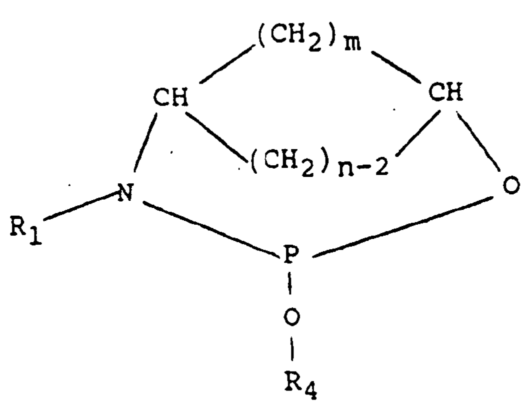
4. Composé selon la revendication 3, dans lequel R_4 représente méthyle.

5. Composé selon l'une quelconque des revendications 1 à 4, dans lequel R_1 représente trifluoroacétyle, (sulfo-2)-fluorényl-9 méthyl carbamate ou fluorényl-9 méthyl carbamate.

6. Méthoxy-2 trifluoroacétyl-3 oxazaphospha-1,3,2 cyclopentane.

7. Méthoxy-2 trifluoroacétyl-3 oxazaphospha-1,3,2 cyclohexane.

8. Composé bicyclique représenté par la formule :



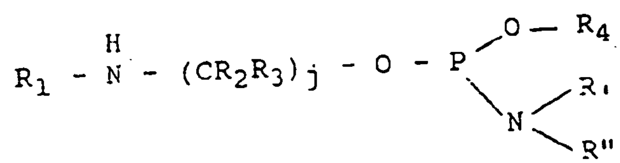
dans laquelle :
- m vaut 1, 2 ou 3 ;

- n vaut 2 ou 3 ;
- R_1 représente trihaloacétyle, acétoacétyle, fluorénylméthyl carbamate ou trityle ou trityle substitué par alcoxy en C_1 à C_3 ;
- R_4 représente alkyle, alcényle, aryle, aralkyle ou cycloalkyle contenant jusqu'à 10 atomes de carbone.

9. Composé selon la revendication 8, dans lequel R_4 a la signification spécifiée à la revendication 3.

10. Composé selon la revendication 8 ou la revendication 9, dans lequel R_1 a la signification spécifiée à la revendication 5.

11. Composé représenté par la formule :



dans laquelle :
- j est un nombre entier de 2 à 10 ;
- R_1 est un groupe de protection d'amino ;
- R_2 et R_3 représentent chacun hydrogène ; alkyle en C_1 à C_6 ; alkyle en C_1 à C_6 substitué par mono-, di- ou trihalométhyle, halo, cyano, sulfo ou nitro ; cyano, halo ou nitro ; et
- R_4 représente alkyle, alcényle, aryle, aralkyle ou cycloalkyle contenant jusqu'à 10 atomes de carbone ;
- R' et R" représentent chacun alkyle, aryle, aralkyle, cycloalkyle, ou cycloalkylalkyle contenant jusqu'à 10 atomes de carbone ; ou
- R' et R" forment ensemble une chaîne alkylène contenant jusqu'à 5 atomes de carbone dans la chaîne principale et un total allant jusqu'à 10 atomes de carbone, les deux liaisons de valence terminales de la chaîne étant attachées à l'atome d'azote auquel R' et R" sont attachés ; ou bien R' et R", conjointement avec l'atome d'azote auquel ils sont attachés, forment un hétérocycle azoté saturé.

12. Composé selon la revendication 11, dans lequel
- R_1 représente trihaloacétyle, acétoacétyle ou fluorénylméthyl carbamate ; et
- R_4 représente alkyle en C_1 à C_6 ; éthyle substitué par bêta-trihalométhyle, bêta-nitro, bêta-sulfo ou bêta-cyano ; phényle substitué par halo, nitro, sulfo ou cyano ; ou phényléthyle substitué par halo, nitro, sulfo ou cyano.

13. Composé selon la revendication 12, dans lequel R_1 représente trifluoroacétyle, (sulfo-2)fluorényl- 9 méthyl carbamate ou fluorényl-9 méthyl carbamate.

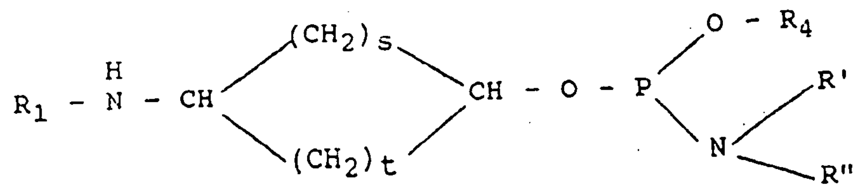
14. Composé selon l'une quelconque des revendications 11 à 13, dans lequel R' et R" représentent chacun propyle, isopropyle, isobutyle, sec.-butyle ou tert.-butyle, ou bien R' et R", conjointement avec l'atome d'azote auquel ils sont attachés, représentent morpholino, pyrrolidino, ou pipéridino.

15. Composé selon la revendication 14, dans lequel R' et R" représentent chacun isopropyle, ou bien R' et R", conjointement avec l'atome d'azote auquel ils sont attachés, représentent morpholino.

16. Composé selon la revendication 14 ou la revendication 15, dans lequel R_1 représente trityle ou trityle substitué par alcoxy en C_1 à C_3.

17. Composé selon la revendication 16, dans lequel R_1 représente monométhoxy-4 trityle ou diméthoxy-4,4' trityle.

18. Cycloalcane représenté par la formule :



dans laquelle :

- s se situe dans la plage de 0 à 8 ;
- t se situe dans la plage de 0 à 8 ; et
- s+t se situe dans la plage de 1 à 8 ;
- R_1 représente un groupe de protection d'amino ;
- R_4 représente alkyle, alcényle, aralkyle ou cycloalkyle contenant jusqu'à 10 atomes de carbone ;
- R' et R" représentent chacun alkyle, aryle, aralkyle, cycloalkyle ou cycloalkylalkyle contenant jusqu'à 10 atomes de carbone, ou bien R' et R", conjointement avec l'atome d'azote auquel ils sont attachés, représentent morpholino, pyrrolidino ou pipéridino.

19. Composé selon la revendication 18, dans lequel

- R_1 représente trihaloacétyle, acétoacétyle ou fluorénylméthyl carbamate ; et
- R_4 représente alkyle en C_1 à C_6, éthyle substitué par bêta-trihalométhyle, bêta-nitro, bêta-sulfo ou bêta-cyano ; phényléthyle substitué par halo, nitro, sulfo ou cyano.

20. Composé selon la revendication 18, dans lequel R_1 représente trityle ou trityle substitué par alcoxy en C_1 à C_3.

21. Composé selon la revendication 20, dans lequel R_1 représente monométhoxy-4 trityle ou diméthoxy-4,4' trityle.

22. Composé représenté par la formule :

$$\begin{bmatrix} R_1 \\ H \end{bmatrix} - \overset{H}{N} - (CR_2R_3)_j - O - \underset{\left[\overset{\|}{O}\right]_i}{\overset{\overset{[R_4]_k}{|}}{\overset{O}{\overset{|}{P}}}} - O - [W]$$

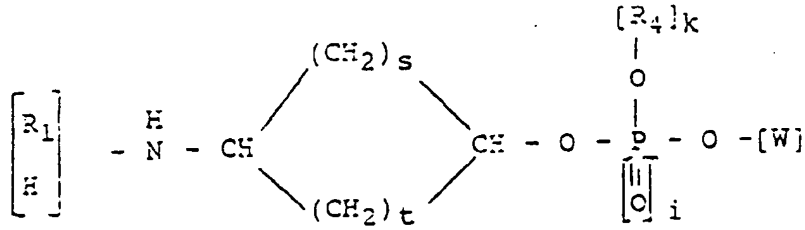
dans laquelle :

- i vaut 0 ou 1 ;
- j est un nombre entier de 2 à 10 ;
- k vaut 1 chaque fois que i vaut 0, ou bien k vaut 0 ou 1 chaque fois que i vaut 1 ;
- R_1 représente un groupe de protection d'amino ;
- R_2 et R_3 représentent chacun hydrogène ; alkyle en C_1 à C6 ; alkyle en C_1 à C_6 substitué par mono-, di- ou trihalométhyle, halo, cyano, sulfo ou nitro ; cyano, halo ou nitro ;
- R_4 représente alkyle, alcényle, aryle, aralkyle ou cycloalkyle contenant jusqu'à 10 atomes de carbone ; et
- W représente un oligonucléotide, un support de polymère, ou un oligonucléotide lié à un support de polymère.

23. Composé selon la revendication 22, dans lequel :

- R_2 et R_3 représentent chacun hydrogène ; et
- R_4 représente alkyle en C_1 à C_6 ; éthyle substitué par bêta-trihalométhyle, bêta-nitro, bêta-sulfo ou bêta-cyano ; phényle substitué par halo, nitro, sulfo ou cyano ; ou phényléthyle substitué par halo, nitro, sulfo ou cyano.

24. Composé cyclique représenté par la formule :



dans laquelle :

- R_1 représente un groupe de protection d'amino ;
- s se situe dans la plage de 0 à 8 ;
- t se situe dans la plage de 0 à 8 ; et
- s + t se situent dans la plage de 1 à 8 ;
- R_4 représente alkyle en C_1 à C_6 ; éthyle substitué par bêta-trihalométhyle, bêta-nitro, bêta-sulfo ou bêta-cyano ; phényle substitué par halo, nitro, sulfo ou cyano ; ou phényléthyle substitué par halo, nitro, sulfo ou cyano ;
- k vaut 1 chaque fois que i vaut 0, ou bien k vaut 0 ou 1 chaque fois que i vaut 1 ; et
- W est un oligonucléotide, un support de polymère, ou un oligonucléotide lié à un support de polymère.

25. Procédé pour synthétiser des oxazaphospha-1,3,2 cycloalcanes substitués en position 2 par alcoxy inférieur et protégés en position 3, le procédé comprenant les étapes consistant à :

- obtenir un précurseur de phosphoramidite tel que défini à la revendication 11 ou 18 ;
- chauffer le précurseur de phosphoramidite pour former un mélange réactionnel contenant un oxazaphospha-1,3,2 cycloalcane substitué en position 2 par alcoxy inférieur et protégé en position 3 ; et
- séparer du mélange réactionnel l'oxazaphospha-1,3,2 cycloalcane substitué en position 2 par alcoxy inférieur et protégé en position 3.

26. Procédé selon la revendication 25, dans lequel le précurseur de phosphoramidite est obtenu par :

- réaction d'une phosphine substituée par halo, par amino N,N-disubstitué et par alcoxy inférieur, avec une alcool amine protégée sur amino, dans un solvant aprotique, pour former un premier mélange réactionnel contenant le précurseur de phosphoramidite ; et
- séparer le précurseur de phosphoramidite du premier mélange réactionnel.

27. Procédé selon la revendication 25 ou la revendication 26, dans lequel l'étape de chauffage comprend un chauffage à une température se situant dans la plage de 25-250 °C.

28. Procédé selon l'une quelconque des revendications 25 à 27, dans lequel l'étape de séparation de l'oxazaphospha-1,3,2 cycloalcane substitué en position 2 par alcoxy inférieur et protégé en position 3 comprend une distillation.

29. Procédé selon l'une quelconque des revendications 25 à 28, dans lequel l'oxazaphospha-1,3,2 cycloalcane substitué en position 2 par alcoxy inférieur et protégé en position 3 est le méthoxy-2 trifluoroacétyl-3 oxazaphospha-1,3,2 cyclopentane ou le méthoxy-2 trifluoroacétyl-3 oxazaphospha-1,3,2 cyclohexane.

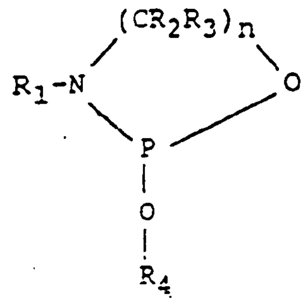
30. Procédé de marquage d'un oligonucléotide, comprenant les étapes consistant à :

- faire réagir un composé tel que défini à la revendication 1, 8, 11 ou 18 avec un hydroxyle non protégé de l'oligonucléotide pour former un conjugué linker - oligonucléotide, le conjugué linker - oligonucléotide ayant une amine protégée ;
- déprotéger l'amine protégée ; et
- faire réagir un marqueur avec l'amine déprotégée.

31. Procédé selon la revendication 30, comprenant en outre l'étape de synthèse de l'oligonucléotide par une synthèse en phase solide, et dans lequel l'étape de réaction du composé tel que défini à la revendication 1, 8, 11 ou 18 est accomplie sous la forme d'une étape d'addition finale dans la synthèse en phase solide.

Patentansprüche

1. Verbindung der Formel



worin
n die Zahl 2, 3 oder 4 bedeutet;
R_1 einen Trihalogenacetylrest oder eine Acetoacetyl-, Fluorenylmethylcarbamat-, Trityl- oder C_1- bis C_3-alkoxy-substituierte Tritylgruppe bedeutet;
R_2 und R_3 jeweils ein Wasserstoffatom, einen C_1- bis C_6-Alkylrest, einen mono-, di- oder trihalogenmethyl-, halogen-, cyan-, sulfo- oder nitrosubstituierten C_1- bis C_6-Alkylrest, eine Cyangruppe, ein Halogenatom oder eine Nitrogruppe bedeuten; und
R_4 einen Alkyl-, Alkenyl-, Aryl-, Aralkyl- oder Cycloalkylrest mit bis zu 10 Kohlenstoffatomen bedeutet.

2. Verbindung nach Anspruch 1, worin n die Zahl 2 oder 3 ist.

3. Verbindung nach Anspruch 1 oder 2, worin R_4 einen C_1- bis C_6-Alkylrest, eine ß-trihalogenmethyl-, ß-nitro-, ß-sulfo- oder ß-cyansubstituierte Ethylgruppe, eine halogen-, nitro-, sulfo- oder cyansubstituierte Phenylgruppe oder eine halogen-, nitro-, sulfo- oder cyansubstituierte Phenylethylgruppe bedeutet.

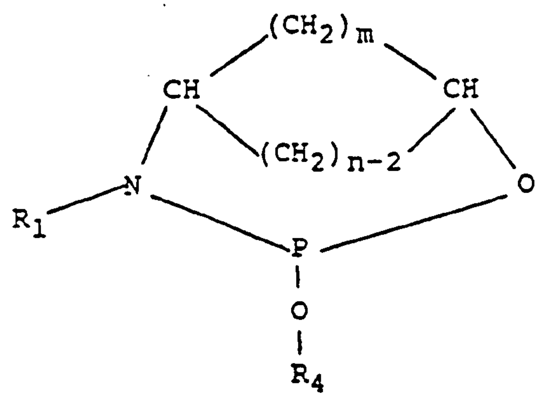
4. Verbindung nach Anspruch 3, worin R_4 eine Methylgruppe bedeutet.

5. Verbindung nach einem der Ansprüche 1 bis 4, worin R_1 eine Trifluoracetyl, 9-(2-Sulfo)-fluorenylmethylcarbamat- oder 9-Fluorenylmethylcarbamatgruppe ist.

6. 2-Methoxy-3-trifluoracetyl-1,3,2-oxazaphosphacyclopentan.

7. 2-Methoxy-3-trifluoracetyl-1,3,2-oxazaphosphacyclohexan.

8. Eine bicyclische Verbindung der Formel

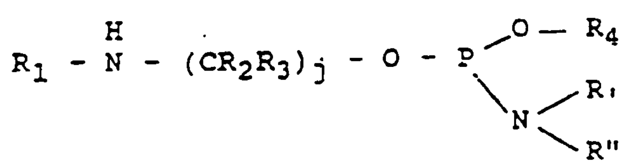


worin
m die Zahl 1, 2 oder 3 ist;
n die Zahl 2 oder 3 ist;
R_1 einen Trihalogenacetylrest oder eine Acetoacetyl-, Fluorenylmethylcarbamat-, Trityl- oder C_1- bis C_3-alkoxysubstituierte Tritylgruppe ist; und
R_4 ein Alkyl-, Alkenyl-, Aryl-, Aralkyl- oder Cycloalkylrest mit bis zu 10 Kohlenstoffatomen ist.

9. Verbindung nach Anspruch 8, worin R_4 die in Anspruch 3 angegebene Bedeutung hat.

10. Verbindung nach Anspruch 8 oder 9, worin R_1 die in Anspruch 5 angegebene Bedeutung hat.

11. Verbindung der Formel



worin
j eine ganze Zahl von 2 bis 10 ist;
R_1 eine Aminoschutzgruppe ist;
R_2 und R_3 jeweils ein Wasserstoffatom, einen C_1- bis C_6-Alkylrest, einen mono-, di- oder trihalogenmethyl-, halogen-, cyan-, sulfo- oder stickstoffsubstituierten C_1-bis C_6-Alkylrest, eine Cyangruppe, ein Halogenatom oder eine Nitrogruppe bedeuten; und
R_4 einen Alkyl-, Alkenyl, Aryl-, Aralkyl- oder Cycloalkylrest mit bis zu 10 Kohlenstoffatomen bedeutet;
R' und R" jeweils einen Alkyl-, Aryl-, Aralkyl-, Cycloalkyl- oder Cycloalkylalkylrest mit bis zu 10 Kohlenstoffatomen bedeutet; oder
R' und R" zusammen eine Alkylenkette mit 5 Kohlenstoffatomen in der Hauptkette und einer Gesamtzahl von bis zu 10 Kohlenstoffatomen bilden, wobei Endvalenzbindungen der Kette an das Stickstoffatom gebunden sind, an welches R' und R" gebunden sind; oder R' und R" zusammen mit dem Stickstoffatom, an das sie gebunden sind, einen gesättigten Stickstoffheterocyclus bilden.

12. Verbindung nach Anspruch 11, worin R_1 einen Trihalogenacetylrest oder eine Acetoacetyl- oder Fluorenylmethylcarbamatgruppe und R_4 einen C_1- bis C_6-Alkylrest oder eine ß-trihalogenmethyl, ß-nitro-, ß-sulfo- oder ß-cyansubstituierte Ethylgruppe oder eine halogen, nitro-, sulfo- oder cyansubstituierte Phenylethylgruppe bedeuten.

13. Verbindung nach Anspruch 12, worin R_1 eine Trifluoracetyl-, 9-(2-sulfo)-Fluorenylmethylcarbamat- oder 9-Fluorenylmethylcarbamatgruppe bedeutet.

14. Verbindung nach einem der Ansprüche 11 bis 13, worin R' und R" jeweils eine Propyl-, Isopropyl-, Isobutyl-, sec.-Butyl- oder tert.-Butylgruppe darstellen oder R' und R" zusammen mit dem Stickstoff-

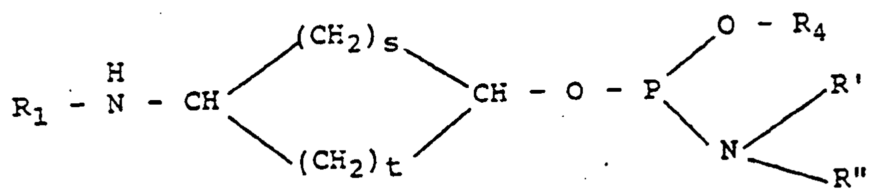
atom, an das sie gebunden sind, Morpholin, Pyrrolidon oder Piperidin bilden.

15. Verbindung nach Anspruch 14, worin R' und R" jeweils eine Isopropylgruppe oder R' und R" zusammen mit dem Stickstoffatom, an das sie gebunden sind, Morpholin bilden.

16. Verbindung nach Anspruch 14 oder 15, worin R_1 eine Trityl- oder C_1- bis C_3-alkoxysubstituierte Tritylgruppe bedeuten.

17. Verbindung nach Anspruch 16, worin R_1 eine 4-Monomethoxytrityl- oder 4,4'-Dimethoxytritylgruppe bedeutet.

18. Cycloalkan der Formel



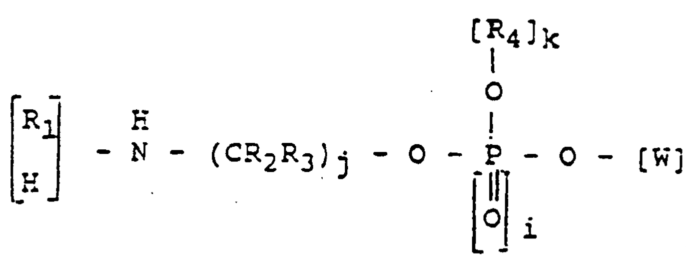
worin
s im Bereich von 0 bis 8, t im Bereich von 0 bis 8, und s + t im Bereich von 1 bis 8 liegt;
R_1 eine Aminoschutzgruppe ist;
R_4 einen Alkyl-, Alkenyl-, Aralkyl- oder Cycloalkylrest mit bis zu 10 Kohlenstoffatomen bedeutet;
R' und R" jeweils einen Alkyl-, Aryl-, Aralkyl-, Cycloalkyl- oder Cycloalkylalkylrest mit bis zu 10 Kohlenstoffatomen bedeuten oder R' und R" zusammen mit dem Stickstoffatom, an das sie gebunden sind, Morpholin, Pyrrolidin oder Piperidin bilden.

19. Verbindung nach Anspruch 18, worin R_1 einen Trihalogenacetylrest oder eine Acetoacetyl- oder Fluorenylmethylcarbamatgruppe bedeutet und R_4 einen C_1- bis C_6-Alkylrest, eine ß-trihalogenmethyl-, ß-nitro-, ß-sulfo- oder ß-cyansubstituierte Ethylgruppe oder eine halogen-, nitro-, sulfo- oder cyansubstituierte Phenylethylgruppe darstellt.

20. Verbindung nach Anspruch 18, worin R_1 eine Trityl- oder C_1- bis C_3-alkoxysubstituierte Tritylgruppe ist.

21. Verbindung nach Anspruch 20, worin R_1 eine 4-Monomethoxytrityl- oder 4,4'-Dimethoxytritylgruppe bedeutet.

22. Verbindung der Formel

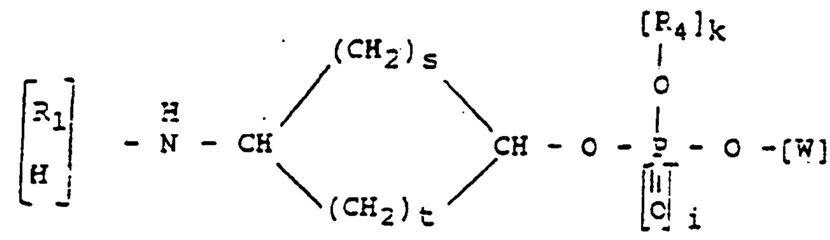


worin
i gleich 0 oder 1 ist;
j eine ganze Zahl von 2 bis 10 ist;
k gleich 1 ist, wenn i gleich 0 ist, oder k gleich 0 oder 1 ist, wenn i gleich 1 ist;
R_1 eine Aminoschutzgruppe bedeutet;
R_2 und R_3 jeweils ein Wasserstoffatom, einen C_1 bis C_6-Alkylrest, einen mono-, di- oder trihalogenmethyl-, halogen-, cyan-, sulfo- oder nitrosubstituierten C_1- bis C_6-Alkylrest, eine Cyangruppe,

ein Halogenatom oder eine Nitrogruppe bedeuten;
R_4 einen Alkyl-, Alkenyl-, Aryl-, Aralkyl- oder Cycloalkylrest mit bis zu 10 Kohlenstoffatomenen bedeutet; und W ein Oligonucleotid, einen Polymerträger oder ein an den Polymerträger gebundenes Oligonucleotid bedeutet.

23. Verbindung nach Anspruch 22, worin R_2 und R_3 jeweils ein Wasserstoffatom bedeuten und R_4 einen C_1- bis C_6-Alkylrest, eine ß-trihalogenmethyl, ß-nitro-, ß-sulfo- oder ß-cyansubstituierte Ethylgruppe, eine halogen-, nitro-sulfo- oder cyansubstituierte Phenylgruppe, oder eine halogen-, nitro-, sulfo- oder cyansubstituierte Phenylethylgruppe bedeuten.

24. Cyclische Verbindung der Formel



worin
R_1 eine Aminoschutzgruppe bedeutet;
s im Bereich von 0 bis 8, t im Bereich von 0 bis 8 und s + t im Bereich von 1 bis 8 liegt;
R_4 einen C_1- bis C_6-Alkylrest, eine ß-trihalogenmethyl-, ß-nitro-, ß-sulfo- oder ß-cyansubstituierte Ethylgruppe, eine halogen-, nitro-, sulfo- oder cyansubstituierte Phenylgruppe oder eine halogen-, nitro-, sulfo- oder cyansubstituierte Phenylethylgruppe bedeutet;
k gleich 1 ist, wenn i gleich 0 ist, oder k gleich 0 ist, wenn i gleich 1 ist; und
W ein Oligonucleotid, einen Polymerträger oder ein an einen Polymerträger gebundenes Oligonucleotid darstellt.

25. Verfahren zum Synthetisieren von 2-niederalkoxy-3-geschützten 1,3,2-Oxazaphosphacycloalkanen, mit folgenden Verfahrenschritten:

Bereitstellen eines Phosphoramiditvorläufers nach Anspruch 11 oder 18;

Erwärmen des Phosphoramiditvorläufers zur Bildung eines Reaktionsgemisches enthaltend ein 2-niederalkoxy-3-geschütztes 1,3,2-Oxazaphosphacycloalkan; und

Abtrennen des 2-niederalkoxy-3-geschützten 1,3,2-Oxazaphosphacylcoalkans vom Reaktionsgemisch.

26. Verfahren nach Anspruch 25, wobei der Phosphoramiditvorläufer hergestellt wird durch:

Umsetzung eines halogensubstituierten N,N-di-substituierten Niederalkoxyphosphins mit einem aminogeschützten Alkoholamin in einem aprotischen Lösungsmittel zur Bildung eines ersten Reaktionsgemisches mit einem Phosphoramiditvorläufer; und

Trennen des Phosphoramiditvorläufers vom ersten Reaktionsgemisch.

27. Verfahren nach Anspruch 25 oder 26, worin die Erhitzungsstufe aus einer Erwärmung auf eine Temperatur im Bereich von 25 bis 250° C besteht.

28. Verfahren nach einem der Ansprüche 25 bis 27, worin die Abtrennung des 2-niederalkoxy-3-geschützten 1,3,2-Oxazaphosphacycloalkans eine Destillation umfaßt.

29. Verfahren nach einem der Ansprüche 25 bis 28, worin das 2-niederalkoxy-3-geschützte 1,3,2-Oxazaphosphacycloalkan das 2-Methoxy-3-trifluoracetyl-1,3,2-oxazaphosphacyclopentan oder das 2-Methoxy-

3-trifluoroacetyl-1,3,2-oxazaphosphacyclohexan ist.

30. Verfahren zum Markieren eines Oligonucleotids, bestehend aus folgenden Verfahrensschritten:

Umsetzen einer Verbindung nach Anspruch 1, 8, 11 oder 18 mit einer ungeschützten Hydroxylgruppe des Oligonucleotids zur Bildung eines Linker-Oligonucleotidkonjugats, welches eine geschützte Aminogruppe enthält; Entfernen der Schutzgruppe von dem geschützten Amin; und Umsetzen eines Markierungsstoffes mit dem ungeschützten Amin.

31. Verfahren nach Anspruch 30, das weiterhin den Schritt einer Synthetisierung des Oligonucleotids durch eine Festphasensynthese umfaßt, worin der Schritt der Umsetzung der Verbindung von Anspruch 1, 8, 11 oder 18 als letzter Zugabeschritt der Festphasensynthese vollständigt durchgeführt wird.